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PHYSIOLOGY.[1]

NOTES ON THE PREPARATION OF NUTRIENT GELATINE AND AGAR.–The practical worker in Bacteriology deplores the loss of time usually attendant upon the preparation, and especially upon the filtration of nutrient gelatine and agar. The method formulated by Koch and closely followed by most workers, is very satisfactory in producing good, clear culture media, but a few modifications render the procedure a much less formidable one, and as the changes to be suggested are simply those of detail, it may be well to state in brief the method now in use in this laboratory, which after considerable trial gives uniform and satisfactory results.

One pound ($\frac{1}{2}$ kg.) of finely chopped beef, as free as possible from fat and gristle, is mixed with 1000 c. c. of distilled water and kept in a cool place for 12 or 18 hours. It is then strained, cold, through a coarse cloth, into a wide-mouthed "agate ware" or "enameled iron" vessel of sufficient size, and 5 gm. of C. P. sodium chloride, 10 gm. of pepton,[1] and 100 gm. of gelatine[2] (or 10 gm. of Agar) are added. This is then placed in a water bath[3] (to which a large handful of rock salt has been added, if agar is to be prepared) and the gelatine (or agar) melted as rapidly as possible. The fluid is then neutralized by the careful addition of sodium bicarbonate in solution, and the boiling continued for a few minutes after, in order to precipitate the phosphates.

The fluid is now cooled by running water, to such a temperature as will not coagulate the white of egg, yet not enough to solidify it, when the whites of two eggs, thoroughly beaten up are mixed with it, and the whole boiled for half an hour.

Filtration which has usually been effected by means of filter paper, can be much more rapidly performed by the use of *absorbent cotton in large quantity*. The pores of the paper become clogged by the fine precipitates and by the cooling of the medium, and even with the use of the "hot funnel" the filtration is sometimes very slow. Cotton, on the other hand, presents in its meshes a much larger surface for the entanglement of the fine precipitates, and when used in large quantity, allows the gelatine (or agar) even when not very hot, to flow through it rapidly. The preparation of the filter is as follows: The absorbent cotton is unrolled, and sterilized in bulk in the hot-air chamber, care being taken not to char it. A six-inch

[1] This department is edited by Prof. Wm. T. Sedgwick, of the Mass. Institute of Technology, Boston, to whom brief communications, books for review, etc., should be sent.

[2] *Comte fils Gelatine premiere qualite*, gives excellent results.

[3] *Pepton Sicc. Extra*, from G. A. Hesterberg, Berlin, is used, as it imparts no color.

[4] The form that has been found most convenient is known as an agate ware "Milk or Rice boiler."

(15 ctm.) glass funnel is packed full with the dry sterilized cotton, placed in in layers, in such a way as to keep it well out of the neck, and having no folds nor ridges of cotton next the glass, through which the precipitates might pass into the receiving flask.

The neutralized culture medium, after being boiled with the white of egg, as above described, is strained through coarse flannel into a flask, and poured slowly upon the centre of the filter until the cotton is thoroughly soaked, and the fluid begins to run into the flask below. This moistening causes the cotton to sink considerably, and packs it in the funnel, and when packed, the fluid filters through it almost as rapidly as it is poured into the funnel. The funnel is now filled and the fluid filtered as fast as it will run through. The first filtration seldom produces a clear medium, but through the same filter the fluid may be poured again and again, each time becoming clearer, and the moderate cooling which necessarily occurs, does not sensibly retard the rapidity of filtration. When filtration is completed, a considerable portion of the medium entangled in the filter can be saved, by pressing upon the cotton with a sterilized glass rod, gently at first and near the sides, then in the centre and with considerable force. The gelatine or agar pressed from the cotton is sometimes cloudy, for which reason it is well to catch it in a separate flask.

It not infrequently happens that gelatine which filters clear precipitates phosphates on boiling; and that agar, on cooling, forms a flocculent precipitate. To insure against filling tubes with such media, it is safest always to fill one tube with the medium, and by first cooling, then by boiling and again cooling, to test the permanence of the transparency obtained. Should these precipitates form, it will be necessary to boil the gelatine in the flask, and to refilter it through a small plug of dry cotton placed in a funnel; while agar should be allowed to completely solidify, when it is again melted and filtered through a small plug of cotton. The media are now ready for tubing and sterilizing in the usual way.

The large quantity of absorbent cotton used and the considerable amount of medium lost, by remaining entangled in the meshes of the cotton (this may amount to 200 c. c. for each of the large cotton filters employed) are unquestionably objections to this method of filtration, but in its favor it may be stated that one filter when properly packed, serves to clear a large quantity of medium, and the great saving of time in filtering, enables one to prepare a large amount of these nutrients at one operation, which may be stored for future use. Futhermore, the "hot funnel" is dispensed with.

The modifications here described may be best appreciated by the fact that they render it possible to prepare within three hours several litres of the above-mentioned culture media.—*T. M. Cheesman, Jr., M.D.* (From the Bacterial Laboratory of Alumni Association of the College of Physicians and Surgeons, New York City.)